

## **The ovipositor of the relic dragonfly *Epiophlebia superstes*: a morphological re-examination (Odonata: Epiophlebiidae)**

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Key words: Odonata, dragonfly, morphology, phylogeny, ovipositor, *Epiophlebia superstes*, Anisozygoptera.

### **ABSTRACT**

The morphology of the endophytic ovipositor in *Epiophlebia superstes* was studied with light and electron microscopy with special emphasis made on the musculature and microsculpture of the exoskeleton. Structural characters are described and illustrated. The musculature of the ovipositor has many similarities with zygopterans. The ovipositor and 10th adominal segment bear groups of setae and campaniform sensilla, which probably contact the plant surface during egg laying. A group of campaniform sensilla on the base of the stylus may be responsible for the zigzag sequence of egg disposition in *E. superstes*. The phylogenetic significance of the microstructure of the ovipositor in *E. superstes* can be evaluated only after a systematic examination of representatives of other ovipositor-bearing Odonata.

### **INTRODUCTION**

Extant Odonata are generally subdivided into three groups, i.e. Zygoptera, Anisoptera, and Anisozygoptera. The latter contains the monotypic relic family Epiophlebiidae, represented currently by two oriental species *Epiophlebia superstes* (Selys, 1889) and *E. laidlawi* Tillyard, 1921 (Silsby 2001). Anisozygopteran dragonflies possess morphological features similar to both the Zygoptera and the Anisoptera, as well as a number of unique peculiarities (Asahina 1954). Thus, the Anisozygoptera are important for clarification of the character polarities and reconstruction of the phylogeny of Odonata.

It must be noted, that the majority of phylogenetic reconstructions of higher-level relationships in Odonata suffer from the absence of a common morphological character system apart from the wing venation (e.g. Pritykina 1980; Bechly 1996; Lohmann 1996; Trueman 1996). Rehn (2003) has presented the most comprehensive cladistic analysis of the relationships in the main lineages of Odonata. He included several non-wing characters in his data analyses, such as head structures, secondary male genitalia, and larval caudal appendages. However, 79 of 122 of Rehn's characters concerned wing morphology. This emphasizes the importance of a search for new phylogenetically informative characters, and the ovipositor is expected to provide such characters (Matushkina 2005).

The two species of *Epiophlebia* as well as all Zygoptera and aeshnid Anisoptera have a cutting ovipositor, used for egg deposition within plant tissues (St. Quentin 1962). Previous studies have described the external morphology and musculature of the ovipositors in some Zygoptera and Aeshnidae (e.g. Matushkina & Gorb 1997; Matushkina 2004). So far the main source of information about the ovipositor of *E. superstes* is the famous work of Asahina (1954). However, given the particular significance of anisozygopterans for phylogenetic studies of Odonata, a more detailed examination of ovipositor structures in *E. superstes* is required, and this is the aim of this study.

## MATERIAL AND METHODS

Three females fixed in 70% ethanol and one air-dried female of *Epiophlebia superstes* were examined. Terminal abdominal segments were dissected in median (three) and frontal (one) planes. The musculature was examined in water under a stereo microscope by manual dissections and subsequent layer-by-layer reconstructions of the muscles. Then cuticle parts were macerated in 10% KOH and examined in glycerin under a stereo microscope. For a SEM study the cuticle parts were washed in distilled water, dehydrated in a graded ethanol series and in acetone, critical point dried (OM CPD 7501), sputtered with gold-palladium (OM-SC7640) and examined with a Zeiss EVO-50 SEM.

### General description of the odonate endophytic ovipositor

The endophytic ovipositor of Odonata is represented by (1) paired cutting 1<sup>st</sup> and 2<sup>nd</sup> valves suitable for substrate penetration; (2) paired large 3<sup>rd</sup> valves which ensheathe the shaft of the ovipositor laterally in its resting position; (3) one basal plate; (4) paired elongated lateral sclerites beside the 3<sup>rd</sup> valves; and (5) one unpaired internal sclerite, connected by two membranous strips with the bases of the 2<sup>nd</sup> valves (Asahina 1954; Pfau 1985; Matushkina & Gorb 1997). The distal edges of the 3<sup>rd</sup> valves bear movable styli, which have been identified as sensory structures in species of *Lestes* (Matushkina & Gorb 2002). Precise homology of structural parts of the ovipositor in Odonata and other insects is discussed by Matushkina (2008). An important morpho-functional feature of the endophytic ovipositor in Odonata is also the bearing edge ("Stützkante" sensu St. Quentin 1962). It constitutes a part of the post-abdomen, by which a female leans against an oviposition substrate during egg laying. All these parts were found in *E. superstes*.

### Abbreviations

aAp: anterior apophysis of V3

Lam: basal plate of ovipositor (lamina valvarum)

M1-M8: muscles of ovipositor

pAp: posterior apophysis of V3

piv: internal sclerite, or posterior intervalvula

St: stylus

V1/V2/V3: first/second/third valves of ovipositor (valvulae 1/2/3)

## RESULTS

**Cutting valves** slightly curved dorsad (Fig. 1). Basal parts of both V1 fused, each externally with a delta-like sharp posteriad-directed outgrowth on its lateral edge. Dorsal margin of each V1 forms a longitudinal groove (aulax), providing a sliding articulation with V2. External surface of each V1 with two oblique serrated ridges in the middle part, spear-shaped laterally serrated apices, and rows of sensilla on the dorsal surface laterad to aulax (Figs 3e-g). Numerous coeloconic and campaniform sensilla scattered on external surface of V1. Internal surface of V1 with a complicated system of longitudinal and oblique ribs in its proximal and distal parts. The ribs on one side match those of the other as a key-and-lock device and probably serve to keep the two halves of V1 together. Bases of both V2 expanded (Fig. 3b). Endoskeleton of each V2 bearing a posteriad-directed process, serving for attachment of muscle M6. Unpaired internal sclerite (piv) compressed laterally and connected to bases of both V2 by membranous cuticle. Expanded base of each V2 with field of short setae externally (Fig. 3b). Rest of V2 surface with numerous sensilla of various shapes. Medial part of V2 with several oblique serrated ridges (Figs 3a, c). Distal part of V2 with lateral flattened laciniated ledges bearing sensilla on its ventral surface (Figs 3a, c). Ventral edges of each V2 forming a longitudinal rail (rachis), being a part of the sliding articulation with aulax of V1. No connection between the dorsal edges of the V2 pair.

**Basal plate of ovipositor (Lam)** represented by a large sclerite, connecting first valves with sternite of S8 and tergite of S9 (Fig. 1). Anterior margin of Lam covered by sternite of S8, which forms a hollow, hook-like protrusion, being slightly bi-lobed apically. External lateral margins of basal plate with broad lobes. Endoskeletal paired lateral apophyses serve as attachment points of muscle M2 (Figs 2a, b). Anterior edge of Lam strongly concave between apophyses. Posterolateral part of Lam represented by paired anterior parts of gonangulum (Fig. 2b). Its anterior edges connected to either side of Lam, whereas the ventral (primarily posterior) and the dorsal (primarily anterior) edges are connected with V1 and tergite of S9 respectively. Origin of elongated sclerite on lateral part of gonangulum connecting V3 with anterior carina of 9th tergite.

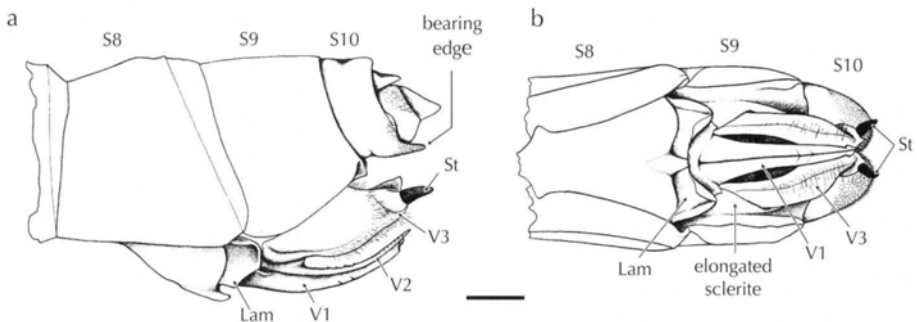
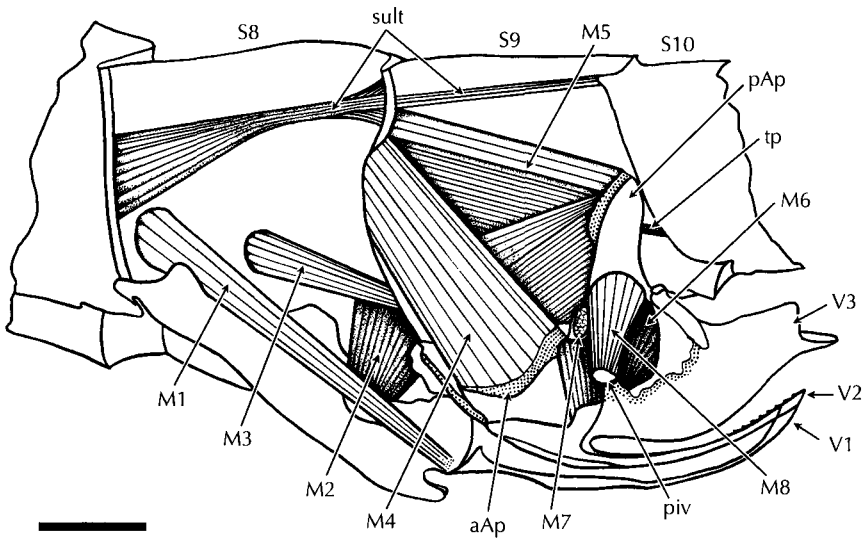


Figure 1: External morphology of the ovipositor of *Epiophlebia superstes* — (a) lateral view; (b) ventral view. Scale bar: 1 mm.

a



b

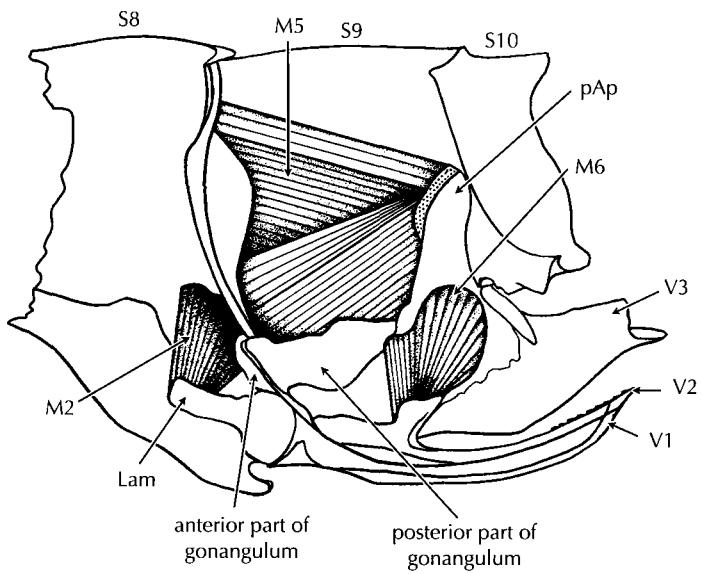


Figure 2: Musculature of the ovipositor of *Epiophlebia superstes*, lateral view — (a) internal layer; (b) external layer; sult, tp: intersegmental abdominal muscles. Scale bar: 1 mm.

**Sheathing valves** (V3) densely covered with setae of various sizes and shapes (Fig. 4c). Endoskeletal part of each V3 possesses two apophyses, aAp and pAp, bearing attachments of muscles M4 and M5 (Figs 2a, b). External surface of each V3 with longest and thinnest setae laterally, whereas the ventral edge of each V3 is covered with short and robust ones. A field of campaniform sensilla (Figs 4c, d) just above the ventral edge near to St. Each sensillum resembling dark spot on pale background of surrounding membranous cuticle. Distal edge of V3 with St bordered with membranous cuticle. Sclerotized protuberance on ventral edge of V3 abutting ventrad to St and bearing short robust setae (Figs 4c, g). St somewhat leaf-shaped, flattened in anteroposterior plane (Fig. 4a). Surface of St entirely covered with short setae, but apex with longer setae forming bunch on ventromedial surface (Figs 4c, f). Group of campaniform sensilla on ventral edge of St beside sclerotised protuberance of V3 but single sensilla scattered on rest of St surface (Figs 4c, e). Group of setae medio-dorsally to the field of campaniform sensilla (Figs 4a, b).

**Bearing edge** situated on S10 (Figs 1a, 5a), represented by two broad ventrolateral outgrowths of segment wall. Numerous robust sharp setae directed posteriorly and few round campaniform sensilla between them (Fig. 5c). Setae obviously rigid, with several longitudinal corrugations (Fig. 5d). Paired scaly knoll situated on each outgrowth between setae (Figs 5a, b).

**Musculature** of ovipositor represented by one unpaired and seven paired muscles (Fig. 2). Paired M1 originating on anterior carina of 8th sternite and inserting on Lam beside lateral apophyses via tendon. Paired M2 originating on ventroposterior edge of 8th tergite and inserting on lateral apophysis of Lam. Paired M3 connecting the lateral part of 8th tergite and anterior carina of 9th tergite. It is not an ovipositor muscle in the ordinary sense, since it is not attached to any ovipositor part. Contraction of M3 moves the 9th tergite anteriorad and, together with M4, draws V2 into the abdomen. Paired large muscle M4 originating on anterior carina and lateral wall of 9th tergite and inserting on aAp of V3. Multiple origins of the largest muscle of ovipositor, the paired M5, on different parts of S9 – the anterior carina of 9th tergite, its dorsolateral, lateral and ventral parts, and the elongated sclerite. Muscle fibers of M5 directed to different attachments, nevertheless no distinct bundles of fibers present. Insertion of M5 situated on pAp. Paired M6 originating on V3 beneath pAp and inserting on a process of basal part of V2. M7, the only unpaired muscle of the ovipositor, running transversad and connecting the two V3 near to pAp. Paired M8 attaching on either side of internal sclerite (piv) and inserting on base of pAp of V3.

## DISCUSSION

The endophytic ovipositor varies within the Odonata mainly in its exoskeleton and musculature (Asahina 1954; St. Quentin 1962; Pfau 1985; Matushkina & Gorb 1997; Matushkina 2004). The complete muscle set of the endophytic ovipositor in odonates comprises seven paired muscles and one unpaired muscle as previously recorded in zygopterans (Matushkina & Gorb 1997). Some muscles of the zygo-pteran ovipositor have a tendency to branch, e.g. M6 is divided into two branches in *Bayadera melanopteryx* Ris (Euphaeidae), *Lestes parvidens* Artobolevsky (Lestidae), and *Heteragrion alienum* Williamson (Megapodagrionidae) (Matushkina 2004), and M6 and M8 are branched in *Lestes barbarus* (Fabricius) (Lestidae), *Enallagma*

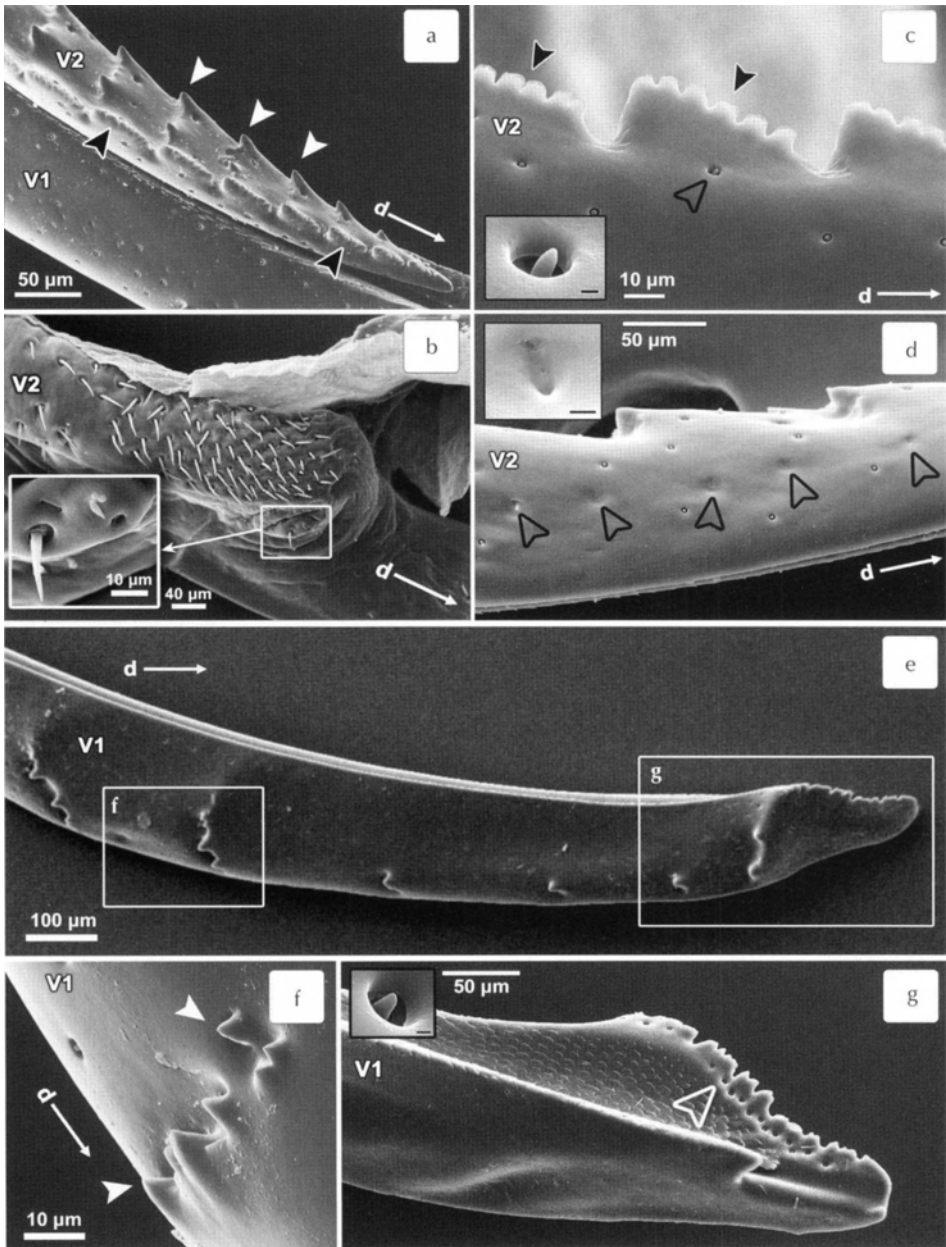


Figure 3: Scanning electron micrographs of the cutting valves of *Epiophlebia superstes* — (a) lateral view of first and second valves; (b) dorsal view of base of second valve; (c) ventral view of second valve in area of lateral ridges – inset shows single sensillum; (d) lateral view of second valve in the middle part – inset shows single campaniform sensillum; (e) lateral view of first valve – insets indicate position of (f) and (g); (f) serrated rib of first valve; (g) internal view of apical part of first valve – inset shows single sensillum. d: distal direction. Arrowheads mark teeth on serration ribs of both valves (white), serrated lateral ridges of second valve (black), and sensilla enlarged in insets (empty). Scale bars of insets in (c), (d), (g): 1 µm.

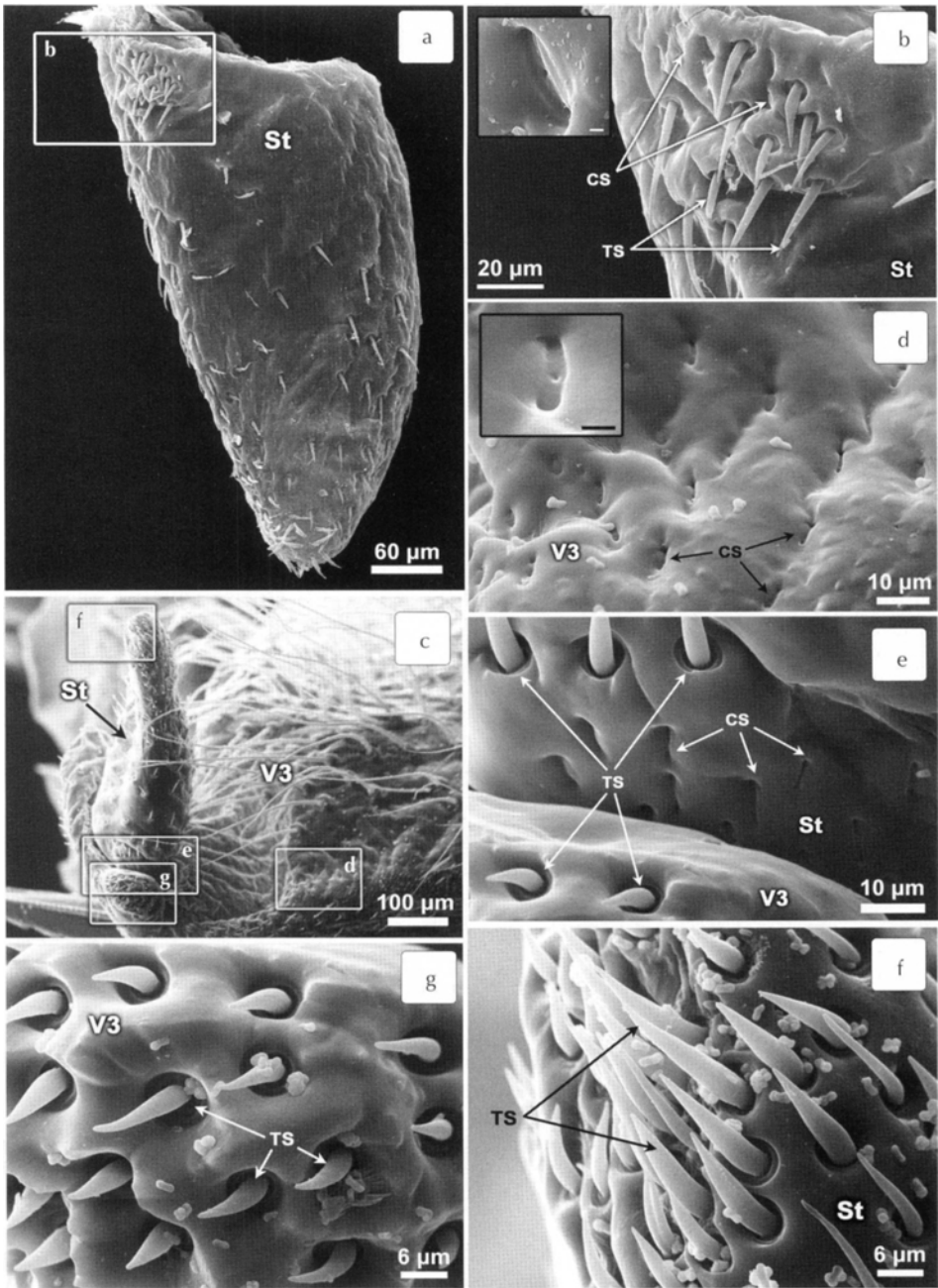


Figure 4: Scanning electron micrographs of sheathing valve and stylus of *Epiophlebia superstes* — (a) internal view of isolated left stylus – inset indicates the group of sensilla on medio-ventral edge; (b) group of sensilla on the stylus – inset shows single campaniform sensillum; (c) ventro-lateral view of terminal area of third valve with stylus – insets mark positions of (d-g); (d) group of campaniform sensilla on sheathing valve – inset shows single sensillum; (e) groups of sensilla on base of stylus and sheathing valve; (f) apex of stylus; (g) sclerotized protuberance with short setae on ventral edge of sheathing valve. CS: campaniform sensilla; TS: hair sensilla. Scale bars: (b, inset) 1 µm; (d, inset) 2 µm.

*cyathigerum* (Charpentier) (Coenagrionidae), *Platycmenis pennipes* (Pallas) (Platynemididae) and *Palaemnema domina* Calvert (Platystictidae) (Matushkina & Gorb 1997; Matushkina 2004). M6 is lost in all aeshnids studied, whereas M5 is divided into two branches (Asahina 1954; Pfau 1985; Matushkina & Gorb 1997). The muscle M6 was not found by Asahina in *Zygoptera*, aeshnids or *Epiophlebia superstes*. This and other facts allowed him to assert that “the female ovipositor is uniformly built throughout the three suborders” (Asahina 1954: 120).

Current knowledge about ovipositor morphology in Odonata suggests that this general conclusion of Asahina (1954) is rather simplistic. The musculature of the ovipositor in *E. superstes* seems to be similar to that in *Calopteryx splendens* (Harris) (Zygoptera, Calopterygidae) (Matushkina & Gorb 1997), since the complete muscle set is present in both species, no muscles are branched, and the attachment sites of homologous muscles are not too different. The sculpture of the cutting valves and bearing edge of the ovipositor correlate with the mechanical properties, mainly with stiffness, of the oviposition substrates (St. Quentin 1962). The position of the bearing edge on S10 is the same as that in aeshnids, although the origin of this structure seems to be different. The bearing edge of *E. superstes* is formed by robust setae instead of teeth or solid processes of S10, which are found in most aeshnids that have been studied (e.g. St. Quentin 1962). Moreover, the sclerotized protuberance with short setae, the robust setae and campaniform sensilla on the sheathing valves of *E. superstes* seem to indicate that this part of the ovipositor also contacts the plant surface during egg laying.

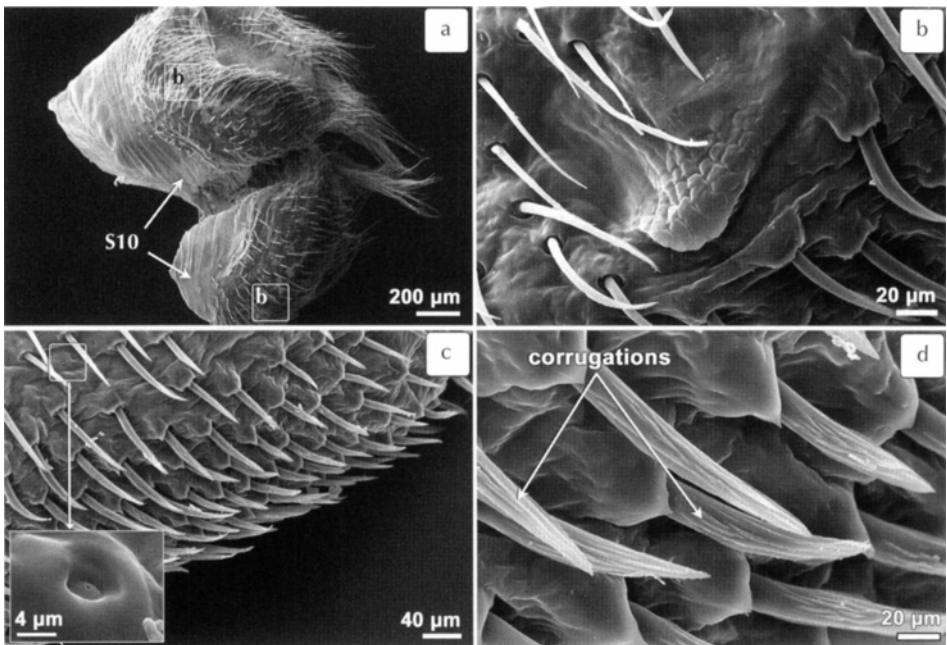


Figure 5: Scanning electron micrographs of S10 of *Epiophlebia superstes* — (a) ventral view of isolated S10, inset indicates position of paired scaly knoll enlarged in (b); (c) robust setae on posterior edge of segment with campaniform sensilla, one of which is shown in inset; (d) sculptures of robust setae.



Asahina stated (1954: 120): "The method of egg laying resembles the Lestid-type, the eggs are inserted into the plant stem standing above the water. The eggs are deposited from below to above." Indeed, the female of *E. superstes* oviposits into the tissues of living plants, placing eggs in zigzag-like rows, which are identical to those in many zygopterans, but resembling coenagrionids or platynemidids rather than lestids (Matushkina & Gorb 2000; Matushkina 2007). However, female lestids move from above to below during oviposition (Jödicke 1997), whereas *E. superstes* females move vice versa. The strikingly regular sequence of egg displacement in *E. superstes* is probably controlled by the group of campaniform sensilla on the base of the stylus (Matushkina & Gorb 2002).

Current evaluations of relationships within Odonata based on morphological and molecular characters propose a sister-group relationship between Epiophlebiidae and Anisoptera, which together form the group Epiprocta (see e.g. discussion in Rehn 2003). However, the morphology of the ovipositor and the egg laying behaviour of *E. superstes* reveal numerous similarities with zygopterans. Furthermore, the ovipositor of *E. superstes* possesses at least one unique character, namely the shape of the stylus, which is leaf-shaped in *E. superstes* in contrast to the cylindrical stylus in other ovipositor-bearing Odonata. Additional phylogenetically informative characters might be found in microstructural features, e.g. position and shape of sensilla and serrations of valves, but this possibility requires a systematic examination of representatives of other ovipositor-bearing Odonata groups.

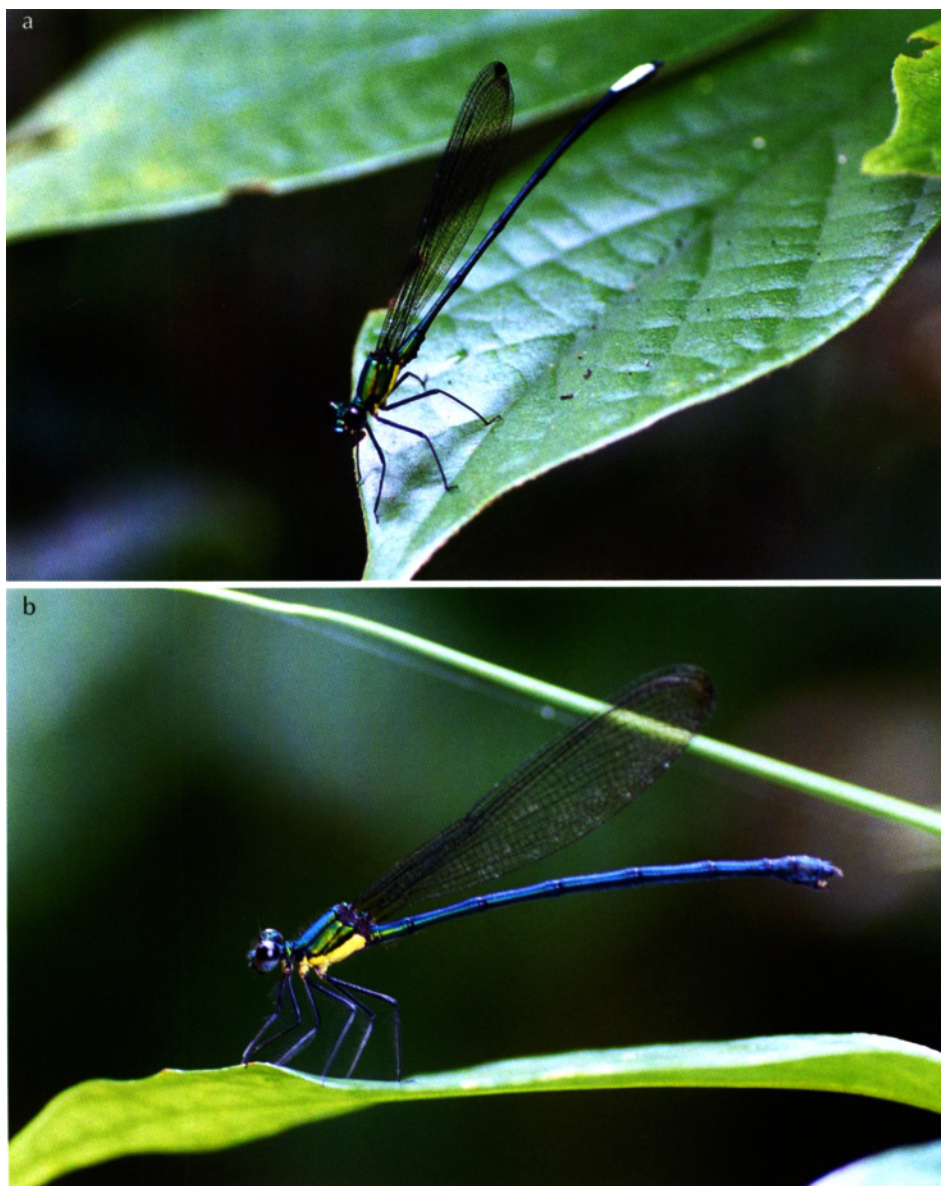
## ACKNOWLEDGEMENTS

Specimens of *Epiophlebia superstes* were kindly provided by Hirozi Naraoka (Kitasugaru-gun, Japan) and Klaus-Dieter Klass (Museum für Tierkunde Dresden). Alex Gumovsky (Schmalhausen Institute of Zoology, Kyiv, Ukraine) and Gordon Pritchard (University of Calgary) helped with the language, and the latter and Stanislav Gorb (MPI for Metals Research, Stuttgart) improved the manuscript. These persons are sincerely thanked. This study was partly supported by German Research Foundation (DFG, 436 UKR 17/27/06).

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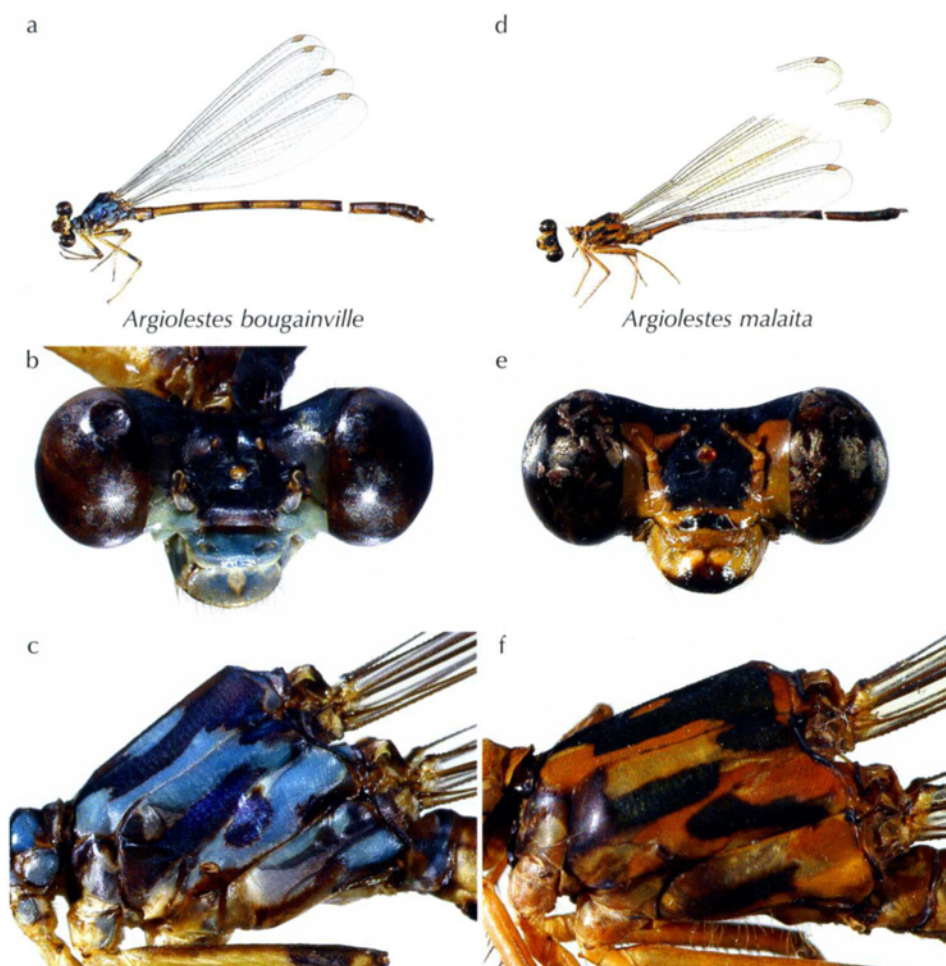


Colour plate I: *Noguchiphaea yoshikoe* — (a) male; (b) female. Silver water fall in Tam Dao mountains, Vinh Phuc Province, northern Vietnam, 30 ix 2007; photo by Do Manh Cuong.



Colour plate II: *Libellago orri* sp. nov. — (a) male, Sg. Penyilam, Bahagian Bintulu, Sarawak, Malaysia, 9 March 2006; (b) presumed female, same location and date. Photos by Rory A. Dow.





Colour plate III: Holotypes of two new *Argiolestes* species — (a-c) *A. bougainville* sp. nov.; (d-f) *A. malaita* sp. nov.; — (a, d) habitus; (b, e) head; (c, f) synthorax.



Colour plate IVa: Male *Ceriagrion tenellum* with a female biting midge (*Forcipomyia paludis*) attached to the right hind wing. Barchetsee, Switzerland, 20 June 2006. Photo by Beat Schneider.



Colour plate IVb: Male of *Phoenicagrion flammeum* in the understory of the Amazon forest at Campamento Junglaven (5°06'N, 66°22'W), Amazonas, Venezuela, where it was common during the dry season, 3 January 2001. Photo by Dennis Paulson.